Résumé de thèse

Obtention, caractérisation et cryoconservation de cellules de nageoire de poisson rouge *Carassius auratus*. Perspectives d'utilisation pour le transfert nucléaire, par Paul-Erwan MAUGER (1)

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La thèse s'inscrit dans la perspective, à moyen terme, d'être capable de régénérer des poissons d'intérêt patrimonial et/ou économique par la voie du transfert nucléaire à partir de tissus somatiques cryoconservés. L'objectif des travaux était de fiabiliser l'obtention et la cryoconservation de cellules somatiques dans la perspective de leur incorporation dans des cryoconservatoires, et dans la perspective d'une étude ultérieure de leur aptitude au transfert nucléaire. Un protocole validé et répétable de culture de cellules de nageoires a été mis au point chez le poisson rouge (*Carassius auratus*). Son application à plusieurs autres espèces de téléostéens appartenant à des genres (*Abramis, Danio, Gobio, Leuciscus, Rutilus*) et des familles phylogénétiquement distantes (Cyprinidae, Salmonidae, Percidae) a été démontrée. En combinant les approches morphologique, immunologique et moléculaire (hybridation *in situ*, PCR quantitative), il a été montré que les cellules cultivées sont exclusivement de type épithélial, et un profil de dédifférenciation a été observé au cours du temps de culture. La cryoconservation des cellules a été optimisée afin de garantir le nombre et la qualité des cellules utilisables au sortir de la cryobanque.

Summary. - Obtaining, characterization and cryopreservation of goldfish (*Carassius auratus*) fin cells in the perspective of nuclear transfer.

The use of somatic cells as nucleus carrier for fish genome cryobanking must be considered when gametes and embryos are not available. It is the case in the wild when the maturational state of the caught fish is uncertain, or in aquaculture for species in which sexual maturation takes several years, or when a dramatic epizooticy requires that the genome is collected quickly before the stock is slaughtered. Somatic cell cryopreservation and fish reconstruction with cloning technology could also be considered for fish bearing a valuable genotype or phenotype, in order to obtain large number of progeny from a single animal. In most cases, it is essential to recover somatic cells from the body without sacrificing the animal, and fin explants are good candidates for this purpose. It is also essential that cryopreserved cells possess a good ability to be used for nuclear transfer. The objective of the study was to explore whether fin cells could be reliably obtained from fin explants, and be cryopreserved in the perspective of later studies on fin cell ability towards nuclear transfer. The study was set up according to three strategies: development of a standardized and reliable method of fin cell culture, characterization of cultured cell type, analysis of fin cell capacity to resist cryopreservation-induced damages. Most of the work was developed on goldfish *Carassius auratus* as a first step before adaptation to other teleost species. Our method of fin cell culture allowed that more than 90% of the tested fins give cultured cells, an essential quality of the method when rare species and samples are to be handled. The culture efficiency was not affected by fish sex or by the fin type. Fin collection could be successfully delayed (3 days) from the fin seeding in culture, thereby allowing that the fin be collected at distance from the cell culture facilities. Application of the fin culture method to several other freshwater species, which belong either to different genus *Abramis, Danio, Gobio, Leuciscus, Rutilus* or to distant families (Cyprinidae, Salmonidae, Percidae), proved that no species-specific adaptation was necessary in our conditions, except for culture temperature. Goldfish cultured cells were characterized with morphological, immunological and molecular approaches (*in situ* hybridization, real time RT-PCR). Cells displayed variable and transient shapes, but they homogeneously belonged to the epithelial type. A tendency to dedifferentiation was observed with culture time. Cryopreservation of goldfish fin cells with dimethyl sulfoxide and sucrose at low freezing rates maintained a high viability (dye exclusion assay) and high proliferation ability of the thawed cells. After seeding of the thawed cells, more than half the population showed successful adhesion. This study is an encouraging basis for genome cryopreservation through somatic cells. The culture and cryopreservation methods set up here are now ready to be used for cryobanking of valuable strains. The characterization of the cultured cells as epithelial type is a first step towards the estimation of the cell ability toward nuclear transfer.

Key words. - Cyprinidae - *Carassius auratus* - Epithelial cells - Somatic cells - Cell culture - DMSO - Genome preservation - Cloning.

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